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Ethanol and lactic acid production as affected by sorghum genotype and location☆

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Abstract

Genotype, environment, location, and their interactions have a significant effect on end-use quality characteristics of grain sorghum (*Sorghum biocolor* (L.) *Moench*). The objective of this research was to study the effect of sorghum genotype and production environment on ethanol and lactic acid production. Eight sorghum varieties from two locations were used. Whole sorghum grain was ground, liquefied, saccharified, and fermented to ethanol using *Saccharomyces cerevisiae* (*S. cerevisae*, ATCC 24860). For lactic acid fermentation, whole ground sorghum grain was liquefied and fermented to lactic acid with *Rhizopus oryzae* NRRL 395; saccharification depended upon native glucoamylase. Results with this limited number of sorghum varieties and locations showed that both sorghum genotype and location had a significant effect on ethanol and lactic acid yields. Variations of 5 and 15% in ethanol and lactic acid yields were observed among the 16 sorghum samples. The effect of location on the fermentation yields was as much as 5% for ethanol and 10% for lactic acid. The effects of variety and location on ethanol and lactic acid production are strongly related to chemical composition and physical properties of grain sorghum samples. Ethanol and lactic acid production increased as starch content increased, whereas the ethanol and lactic acid production decreased as protein content increased. Chemical composition had a greater effect on the ethanol and lactic acid yields than physical properties of the sorghum kernels. The effect of physical properties on ethanol and lactic acid yields was not significant ($P > 0.05$).

Keywords: Fermentation; Ethanol; Lactic acid; Grain sorghum; Variety; Environment

1. Introduction

The use of renewable biomass as a substrate for petrochemical is becoming increasingly important as petroleum supplies continue to decline. The world's leading industries and manufacturers are seeking to replace petrochemical-based feedstock

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with agricultural-based materials. Currently, bioenergy represents about 8% of the total energy used in the US annually. Government-Industry Initiatives such as the 'Plant/Crop-Based Renewable Resources 2020 Vision' have proposed targets for the biobased products industry (National Research Council, 2000). The target is to provide at least 25% of 1994 levels of organic carbon-based industrial feedstock chemicals and 10% of liquid fuels by the year 2020. This would entail a fivefold increase in the use of renewable bio-resources to meet growing demands. These targets have stimulated research into the potential for increased use of biomass. Starch and cellulose could provide the bioresource platform for numerous products.

Ethanol is growing as a 'clean' substitute for direct use as fuel, which can ease both natural resource limitation and reduce environment pollution. Around 6.13 million metric tons of ethanol were produced in 2002, mainly from the fermentation of cornstarch. Lactic acid is commonly used in the food, textile, and chemical industries. Recently, lactic acid has been used as a raw material for polylactic acid (PLA) production. PLA is a biodegradable thermoplastic resin that can be substituted for petroleum-based thermal plastics, reducing environmental pollution and other problems associated with petroleum-based plastics (Kharas et al., 1994). The potential PLA market is about 12 million metric tons annually and represents about 44.1% of the entire thermoplastic resin market (Narayan, 1994). With its multiple uses, there will likely be a rapidly increasing demand for lactic acid in the near future. Lactic acid is the simplest hydroxy acid having an asymmetric carbon atom. It exists in two optical active isomers with opposite rotations of polarized light—D (+) lactic acid and L (+) lactic acid. Only L (+) lactic acid primarily produced by *Rhizopus oryzae* (*R. oryzae*) can be used for PLA production.

Grain sorghum (*Sorghum biocolor* (L.) Moench) is a starch-rich grain and is one of the optimal crops for industrial applications. Sorghum is a tropical grass grown primarily in semiarid and dried parts of the world, especially in areas too dry for maize. Sorghum cannot compete successfully with corn as a cereal in an agroecosystem with over 1000 mm of annual rainfall, but corn cannot replace sorghum in areas with less than 900 mm of rainfall. As great diversity of climate and a limitation of annual rainfall, sorghum is an important cereal crop, especially in the arid area in world. In the US, sorghum is an important crop, especially in the Central Great Plains area. Sorghum production ranks third among cereal crops with about 4 million ha planted in 2000 exceeded only by corn and wheat (USDA, 2002). Sorghum has a similar chemical composition as corn. However, sorghum has been an underutilized material for value-added products and industrial applications. About 95% of sorghum has traditionally been used for feed and only about 5% of sorghum for ethanol production. Due to its limited use and other causes, annual production of grain sorghum declined 24% from 14.9 million metric tons in 1991 to 11.9 million metric tons in 2000 in the US (USDA, 2002). At the same time, corn production increased 33% from 190 to 253 million metric tons. The major barriers affecting sorghum's utilization for industrial applications are its poor wet-milling properties and low digestibility by microorganisms, which is only 95–96% of corn (Leeson and Summers, 1997).

The production of chemicals and biomaterials from renewable biomass faces significant technical and economic challenges at present. Its success depends largely upon the physical and chemical properties of biomass, pretreatment procedures, efficient microorganisms, and processing conditions. For example, extrusion cooking can depolymerize starch, increase free sugar content, increase bioconversion rate, and reduce fermentation time (Linko et al., 1983; Camire and Camire, 1994; Govidasamy et al., 1995; Zhan et al., 2003). Simultaneous saccharification and fermentation technology can be applied to raw agricultural materials, thus eliminating the starch and glucose production steps (Hang, 1989; Philippidis et al., 1993; Lezinou et al., 1994, 1995; Singh et al., 1995; Suresh et al., 1999a,b; Zhan et al., 2003). However, pretreatment and modification of processing procedures may increase production cost. To increase sorghum bioconversion rate, the strategy should focus on identifying the sorghum varieties that have the best fermentation performance and high digestibility. These may be represented by existing sorghum varieties or by improved varieties developed through plant breeding or genetic transformation.

Genotype, environment, location, and their interactions have a significant influence on end-use quality characteristics of grains. For example, wheat protein content, alkaline water retention, and food product quality are strongly influenced by environment, location, and genotype (Bassett et al., 1989). Currently, sorghum research focuses on improving yield, disease resistance, and stability under different environmental conditions. However, our understanding of the effect of sorghum genotype and growth conditions on properties relevant to its industrial applications is very limited. Therefore, the objective of this research was to study the effect of sorghum genotypes and grain production environments on ethanol and lactic acid production. This information will benefit both industry and sorghum breeders by providing scientific information on those factors that may influence the development of sorghum varieties for industrial applications.

2. Materials and methods

2.1. Materials

Eight sorghum hybrids produced by intercrossing four male and two female parent lines were used for this study. The males used in this study consisted of two normal-seeded lines, Tx2737 and Tx435, and two large-seeded lines, Eastin-1 and KS115. The females were common US seed parent lines AWheatland and ATx3042. The hybrids were planted and grown under dry-land conditions at Kansas State University Experiment Stations in Ottawa and Manhattan. Ottawa is located at 38.7° north latitude and 95.2° west longitude with annual rainfall of 939 mm and Manhattan is located at 39.2° north latitude and 96.6° west longitude with annual rainfall of 838 mm during 2000. The samples from Ottawa were produced on a Woodson silt loam and the samples from Manhattan were produced on a Reading silt loam. The nitrogen loading was 80 lb/acre for both locations. Plots were harvested mechanically at physiological maturity. Seed weight was determined by averaging three replicates of 1000 seeds. Seeds were sampled from the bulked grain harvested at each of the two locations. Proximate components including protein, fat, crude fiber, moisture, and ash were determined using AOAC standard methods (AOAC, 1990). Starch content was determined with kits from Megazyme (Bray, Ireland) by AACC Approved Method 76–12 (AACC, 2000). A Single Kernel Characterization System (Model 4100, Perten Instruments, Huddinge, Sweden) was used to determine kernel hardness and kernel size (Pedersen et al., 1996). The chemical composition and physical properties of the sorghum samples are listed in Tables 1 and 2. The sorghum samples were ground separately using mill made by Glen Mill Inc. (Model No. 93.0303, Clifton, NJ).

2.2. Fermentation method for ethanol production

2.2.1. Microorganism

Saccharomyces cerevisiae (*S. cerevisae*, ATCC 24860) was used for ethanol fermentation. The yeast cells were maintained on YPD medium (per l) with 20 g yeast extract, 5 g peptone, 5 g dextrose, and 20 g agar. Yeast cells were precultured for 48 h at 30 °C in an aqueous solution containing 2% glucose, 0.5% peptone, 0.3% yeast extract, 0.1% KH_2PO_4, and 0.05% $MgSO_4 \cdot 7H_2O$ (pH 5.5) with shaker speed of 200 rpm.

2.2.2. Starch liquefaction and saccharification

Termamyl 120 l (0.01 ml α-amylase/g dry starch) was used for sorghum hydrolization. Erlenmeyer flasks (500 ml) with 100 ml medium at pH 5.8 were placed in the temperature controlled water bath shaker at 95 °C with agitation speed of 50 rpm for 45 min. After lowering the temperature to 80 °C, the second Termamyl 120 l (0.01 ml α-amylase/g dry starch) was added and liquefaction was continued for an additional 30 min. The flasks were then cooled to 60 °C and placed into another water bath shaker with controlled temperature at 60 °C. Then, 1 ml amyloglucosidase solution (3000 U/ml) was added into each flask for starch saccharification based on 150 U/g dry starch.

Table 1
Chemical composition of grain sorghum varieties

Location/Variety	Code as	Starch (%)[a]	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Glutamic acid (%)
Manhattan							
Wheatland/KS115	a	65.3	13.76	3.92	2.74	1.59	2.73
SA3042/KS115	b	65.4	14.18	3.83	2.23	1.55	2.93
Wheatland/Eastin 1	c	65.6	13.42	3.69	2.38	1.56	2.75
ASA3042/Eastin 1	d	68.5	13.31	3.44	2.55	1.46	2.69
Wheatland/TX2737	e	69.7	11.76	3.73	2.39	1.51	2.25
ASA3042/TX2737	f	70.4	11.96	3.52	2.39	1.61	2.24
Wheatland/TX435	g	68.6	11.86	3.23	1.33	1.64	2.31
ASA3042/TX435	h	64.1	12.23	3.86	1.26	1.44	2.35
Ottawa							
Wheatland/KS115	a	69.4	11.35	4.09	2.00	1.09	2.20
ASA3042/KS115	b	67.3	11.47	3.56	1.74	1.29	2.34
Wheatland/Eastin 1	c	67.3	11.81	3.41	1.81	1.35	2.13
ASA3042/Eastin 1	d	71.0	11.60	3.41	1.75	1.22	2.29
Wheatland/TX2737	e	68.8	11.30	3.46	1.54	1.38	2.16
ASA3042/TX2737	f	68.3	11.38	3.39	2.10	1.45	2.26
Wheatland/TX435	g	69.8	10.61	3.02	1.97	1.35	2.17
ASA3042/TX435	h	68.4	12.15	3.08	1.61	1.32	2.31

[a] The chemical composition of sorghum was determined based on dry weight base with three replicates.

Table 2
Physical properties of grain sorghum varieties

Location/Variety	Code as	Kernel hardness (Index)[a]	Kernel diameter (mm)	Kernel weight (mg)[b]
Manhattan				
Wheatland/KS115	a	55.39	2.80	47.94
ASA3042/KS115	b	56.16	2.78	45.79
Wheatland/Eastin 1	c	72.48	2.37	32.06
ASA3042/Eastin 1	d	69.88	2.51	34.18
Wheatland/TX2737	e	78.29	2.16	27.07
ASA3042/TX2737	f	76.25	2.25	27.10
Wheatland/TX435	g	80.69	2.35	29.52
ASA3042/TX435	h	90.06	2.34	29.31
Ottawa				
Wheatland/KS115	a	55.29	2.62	41.34
ASA3042/KS115	b	59.00	2.64	41.12
Wheatland/Eastin 1	c	71.13	2.27	30.48
ASA3042/Eastin 1	d	62.96	2.22	28.14
Wheatland/TX2737	e	73.54	2.10	26.3
ASA3042/TX2737	f	72.81	2.22	27.18
Wheatland/TX435	g	81.90	2.31	28.45
SA3042/TX435	h	82.90	2.23	26.05

[a] Kernel harness, kernel diameter, and kernel weight were measured by single kernel characteristic system (SKCS) and the data reported were based on average of 300 kernels.

[b] Kernel weight was based on real kernel weight (wet base).

The flasks were held for 30 min at 60 °C with continuous agitation in the water bath shaker at 50 rpm.

Erlenmeyer flasks (500 ml) with 100 ml fermentation medium containing (per l): 200 g ground sorghum substrates, 3 g peptone, 1 g KH_2PO_4, and 1 g $(NH_4)_2SO_4$ at pH 3.8. The peptone and minerals were added before liquefaction as solids. The medium was inoculated with 6% yeast suspension (1×10^6 cells/ml) and incubated in a rotary shaker (200 rpm) for 72 h at 30 °C. All experiments were replicated three times and the average values reported.

2.3. Fermentation method for lactic acid production

2.3.1. Microorganism

Rhizopus oryzae NRRL 395, obtained from the Northern Regional Research Center, USDA-ARS, Peoria, IL, was used for L (+) lactic acid fermentation. Cultures were maintained and spores were produced on potato dextrose agar (PDA) plates. Spores were grown on the PDA plates at 30 °C for 6–8 days. The spores were then collected by washing the plates with sterile water. A spore suspension of 10^7 spores/ml was used for preculture.

2.3.2. Culture medium

Composition of the preculture medium (per l) was: 80 g glucose, 2 g urea, 0.6 g KH_2PO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.088 g $ZnSO_4 \cdot 7H_2O$, and 30 g $CaCO_3$. The medium for lactic acid production consisted (per l) of 150 g ground grain sorghum, 2 g urea, 0.6 g KH_2PO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.088 g $ZnSO_4 \cdot 7H_2O$, and 30 g $CaCO_3$. These media were sterilized at 120 °C for 30 min. Before sterilization, the sorghum was liquefied by addition of 0.2% Termamyl (Novo Inc., Franklinton, NC) at 95 °C for 10 min.

2.3.3. Fermentation

To produce seed culture, a 500-ml flask containing 100 ml of the preculture medium was inoculated with the spore suspension of 10^7 spores/ml and fermented at 33 °C for 16–18 h in a rotary shaker at 240 rpm.

A 500-ml flask containing 100 ml of the production medium was inoculated with 10 ml of the resultant seed culture and fermented at 33 °C in the rotary shaker at 240 rpm. To maintain pH, 5 g of sterilized $CaCO_3$ was added to each flask after 24 h of fermentation. The total fermentation period was 72 h. All experiments were replicated three times and the average values reported.

2.4. Analysis methods

Lactic acid concentration was determined by EDTA Titrimetric Method (Barnard, 1956, 1957). Ethanol concentration was determined by specific gravity method (AOAC-9.017, 1990). Ethanol and lactic acid were also analyzed using HPLC system (Shimadzu DGU-14A) with an Aminex HPX-87H column (Bio-Rad, Hercules, CA) to compare the results with above two methods. The mobile phase was 5 mM H_2SO_4 pumped at a flow rate of 0.6 ml/min. Data acquisition and analysis were performed using the SHIMADZU EZSTART 7.1.1 software. The results reported were based on EDTA Titrimetric Method and specific gravity method. Analysis of variance (ANOVA) and least significant difference were done using ASA (SAS, 1995; SAS, Institute, Cary, NC).

3. Results and discussion

3.1. Effect of sorghum genotype and location on ethanol and lactic acid production

Yields of ethanol and lactic acid were significantly affected by genotype and environmental conditions (Tables 3 and 4). The highest and the lowest ethanol yields at Manhattan site were 8.40% (v/v) and 8.05% (v/v), respectively (Table 3). The difference in ethanol production between the highest and lowest yields was about 4%. The highest and lowest lactic acid yields at Manhattan site were 30.8 and 27.3 g/l, respectively (Table 3). The difference in lactic acid production between the highest and lowest yields was more than 15%. For the Ottawa site, the highest ethanol yields was 8.46% (v/v) and the lowest was 8.25% (v/v). The difference in ethanol production between highest

Table 3
Lactic acid and ethanol production from different grain sorghum varieties and locations

Location/Variety	Code as	Lactic acid yield		Ethanol yield	
		Yield (g/l)	Conversion rate[a]	Yield (% v/v)	% of theoretical[b]
Manhattan					
Wheatland/KS115	a	28.6	29.2	8.18	87.9
ASA3042/KS115	b	[27.3][c]	27.8	8.13	87.2
Wheatland/Eastin 1	c	28.5	29.0	[8.05]	86.1
ASA3042/Eastin 1	d	30.5	29.7	8.06	82.5
Wheatland/TX2737	e	28.6	27.3	8.29	83.4
ASA3042/TX2737	f	(30.8)[d]	29.1	8.39	83.6
Wheatland/TX435	g	30.0	29.2	8.36	85.5
ASA3042/TX435	h	[27.3]	28.3	(8.40)	91.8
Ottawa					
Wheatland/KS115	a	31.8	30.5	8.42	85.1
ASA3042/KS115	b	29.6	29.3	8.44	88.0
Wheatland/Eastin 1	c	29.8	29.5	8.35	82.5
ASA3042/Eastin 1	d	[32.6]	28.2	[8.25]	80.9
Wheatland/TX2737	e	31.0	30.0	8.43	86.0
ASA3042/TX2737	f	30.5	29.8	8.39	86.2
Wheatland/TX435	g	32.3	30.8	(8.46)	85.0
ASA3042/TX435	h	(29.1)	28.3	8.43	86.5

[a] Lactic acid conversion rate = (lactic acid yield (g)/starch (g)) × 100.
[b] Percentage of theoretical ethanol yield = (ethanol yield (g)/(glucose (g) × 0.51)) × 100.
[c] () = Highest yield.
[d] [] = Lowest yield.

Table 4
ANOVA for effect of genotype and location on ethanol and lactic acid production

Varieties	Ethanol		Lactic acid	
	R^2	Pr > F	R^2	Pr > F
Variety		> 0.001		> 0.001
Location		> 0.001		> 0.001
Variety × Location		> 0.001		0.058
Model	0.98	> 0.001	0.92	> 0.001

and lowest was 3%. The highest lactic acid yield was 32.6 g/l and the lowest was 29.1 g/l. The difference between the highest and lowest was about 12%. The optimal variety with high ethanol yield will significantly increase ethanol production and utilization efficiency of the land. With current processing technology, it takes ~13.5 lbs grain starch to make 1 gallon ethanol. If 5% increment in ethanol production using an optimal variety, the ethanol production will increase 9 gal per acre from 185 to 194 gal (yield = 65 bu/acre, starch at 70%). This economic advantage could benefit both producers and industries. If combining two locations together, the difference in ethanol yields between the highest (8.46% (v/v)) and lowest (8.05% (v/v)) was about 5%, and the difference in lactic acid yields between the highest (32.6% g/l) and the lowest (27.3% g/l) was about 19%.

There was a significant variety and location interaction for both the ethanol and lactic acid yields (Table 4). The models with coefficients of determination (R^2) of 0.98 for ethanol and 0.92 for lactic acid were also significant at statistical level of 0.05. We compared the chemical composition and physical properties at average of the two highest and lowest ethanol and lactic acid yields. Varieties with the highest ethanol and lactic acid yields were higher in starch content and lower in protein content, fiber content, glutamic acid content, and kernel hardness (Table 5). This indicates that the variety effect on ethanol and lactic acid

Table 5
Comparison of chemical composition and physical properties of grain sorghum varieties with the highest and lowest ethanol and lactic acid production

Products	Yields	Starch (%)	Protein (%)	Fiber (%)	Glutamic acid (%)	Kernel hardness (index)	Kernel diameter (mm)
Ethanol (% v/v)							
High[a]	8.45	68.6	11.04	1.79	2.24	70.6	2.44
Low[b]	8.05	67.1	13.37	2.47	2.72	71.2	2.48
Difference (%)	5.0	+2.3	−17.4	−27.5	−17.6	−0.8	−1.6
Lactic acid (g/l)							
High[a]	32.5	70.4	11.11	1.86	2.23	72.4	2.27
Low[b]	27.3	64.8	13.21	1.76	2.64	73.1	2.56
Difference (%)[c]	19.0	8.6	−18.9	5.7	−18.4	−0.9	−12.7

[a] High = Average of two sorghum samples with the highest fermentation yields.
[b] Low = Average of two sorghum samples with the lowest fermentation yields.
[c] Difference (%) = (High − Low)/Low × 100.

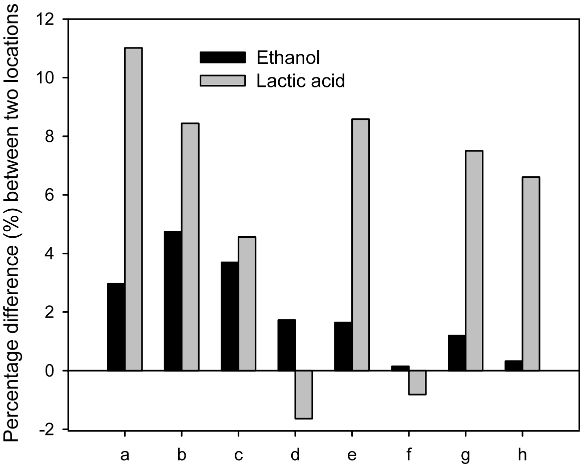

Fig. 1. Effect of grain sorghum growth environment on ethanol and lactic acid production. The difference was calculated based on equation of (Ottawa − Manhattan)/Manhattan × 100.

yields is strongly related to chemical composition and physical properties of sorghum varieties.

Location also affected ethanol and lactic acid yields (Fig. 1). For ethanol fermentation, the environmental effect was less than 5% in terms of ethanol yield for the individual sorghum varieties. The environmental effect on lactic acid fermentation was more than 10% for lactic acid yield. This indicates that grain production environment has a greater influence on lactic acid fermentation than ethanol fermentation. In general, the sorghum produced at the Ottawa location had higher average ethanol and lactic acid yields than the sorghum produced at the Manhattan location. The sorghum samples from Ottawa were higher in starch content, lower in protein, glutamic acid, and fiber contents, higher in kernel hardness, and had smaller kernels than those from the Manhattan location (Table 6). This indicates that environment had a significant effect on chemical composition and physical properties of the sorghum used in this study, which in turn significantly affected ethanol and lactic acid yields.

3.2. Effect of chemical composition of grain sorghum on ethanol and lactic acid production

Chemical composition had a greater effect on the ethanol and lactic acid yields than physical properties of the sorghum kernels. The coefficients of determination (R^2) as a function of major chemical composition of sorghum ($R^2 = 0.88$ for ethanol and $R^2 = 0.78$ for lactic acid) were greater than those as a function of major physical properties ($R^2 = 0.65$ for ethanol and $R^2 = 0.64$ for lactic acid) (Table 7). The effect of physical properties on ethanol and lactic acid yields was not significant ($P > 0.05$). Kernel size did have a significant effect on lactic acid production. This may be an artifact because of the limited number of sorghum varieties tested. The results from Table 5 shows that varieties with low kernel hardness had higher ethanol and lactic acid yields. This result is

Table 6
Effect of location on ethanol and lactic acid (LA) production and major chemical composition and physical properties of sorghum varieties

Location	Ethanol (%v/v)	Lactic acid (g/l)	Starch (%)	Protein (%)	Fiber (%)	Glutamic acid (%)	Kernel hardness (index)	Kernel diameter (mm)
Manhattan	8.23	28.9	67.2	12.81	2.16	2.53	72.43	34.12
Ottawa	8.40	30.9	68.2	11.46	1.82	2.23	69.94	31.13
Difference (%)	2.1	6.9	+2.4	−10.5	−15.9	−11.8	−3.4	−8.7

Table 7
ANOVA for the effect of chemical composition and physical properties of grain sorghum varieties on ethanol and lactic acid production

Varieties	Ethanol		Lactic acid	
	R^2	Pr > F	R^2	Pr > F
Physical properties				
Kernel hardness (X_1)		0.133		0.653
Kernel diameter (X_2)		0.356		0.025
Kernel weight (X_3)		0.602		0.057
X_1X_2		0.261		0.181
X_2X_3		0.075		0.498
X_1X_3		0.074		0.774
$X_1X_2X_3$		0.305		0.837
Model[a]	0.65	0.151	0.64	0.087
Chemical composition				
Starch (X_1)		0.020		0.003
Protein (X_2)		0.003		0.178
Fiber (X_3)		0.001		0.867
X_1X_2		0.829		0.321
X_2X_3		0.770		0.073
X_1X_3		0.738		0.313
$X_1X_2X_3$		0.045		0.151
Model	0.88	0.004	0.78	0.031

[a] $Y = a + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_1X_2 + a_5X_1X_3 + a_6X_2X_3 + a_7X_1X_2X_3$, where Y is ethanol or lactic acid, a is constant, a_n are coefficients, X_1 is kernel hardness or starch, X_2 is kernel diameter or protein, and X_3 is kernel weight or fiber.

expected because in the hard endosperm, starch granules are polygonal and tightly packed together. Protein-starch interactions are thought to be very strong in the corneous endosperm and the starch granules surrounded by a continuous protein matrix with embedded protein bodies. Conversely, in the floury (soft) endosperm, the starch granules are spherical and not tightly packed with a more open structure and starch granules embedded in a discontinuous protein matrix containing fewer protein bodies (Shull et al., 1990). Starch, protein, and fiber content and their interactions all had a significant effect on ethanol production. Only starch had a significant effect on lactic acid production. This was probably because the starch effect had greater weight than the other chemical components in the model. In addition, the mycelia cling together easily and form a large cake during lactic acid fermentation with fungi. The large cake may reduce aeration and nutrient supply during fermentation. Lactic acid fermentation is an aerobic fermentation and the fermentation environment created with the shaker fermentation system was weak in air supply and agitation. These factors may reduce the effect of some chemical components on the lactic acid fermentation in the model tested.

Both starch and protein were strongly correlated with ethanol and lactic acid yields based on regression analysis using single chemical composition. Both ethanol and lactic acid yields increased as starch content increased and decreased as protein content increased (Figs. 2–5). The coefficients of determination (R^2) were 0.12 and 0.55 for the correlation between starch content and ethanol and lactic acid yields (Figs. 2 and 4). The coefficients of determination (R^2) were 0.71 and 0.46 for the correlation between protein content and ethanol and lactic acid yields (Figs. 3 and 5). Protein content may be inversely proportional to starch content. The relationship between protein content and theoretical percentage of ethanol yield indicates that protein content had no significant effect on ethanol yield (Fig. 6). Starch granules are typically imbedded in a protein matrix. Protein bodies of sorghum are composed primarily of prolamins (storage protein), whereas the matrix protein is primarily glutelin (Taylor et al., 1984). Protein content and type of protein should play important role on the fermentation process. The preceding result could be explained on the availability of grain starch as a basic carbon source and type of protein may vary and impact the conversion rate and final product yields. Additional research is needed to understand the effect of starch structure and starch and protein interaction on bioconversion process.

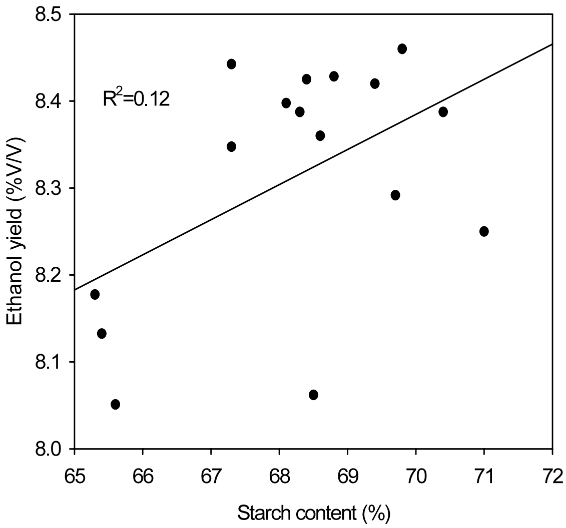

Fig. 2. Effect of starch content on ethanol production.

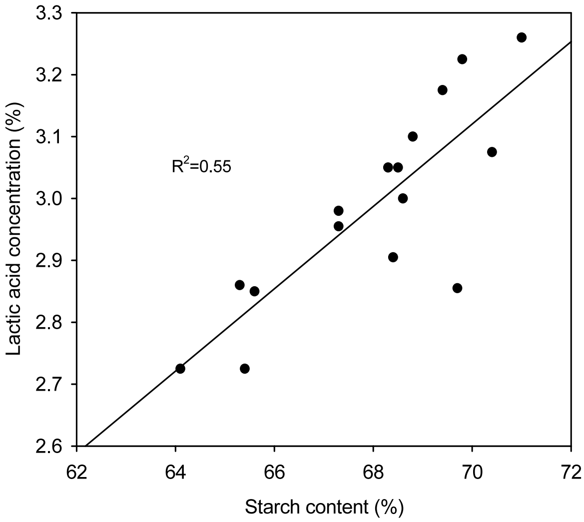

Fig. 3. Effect of starch content on lactic acid production.

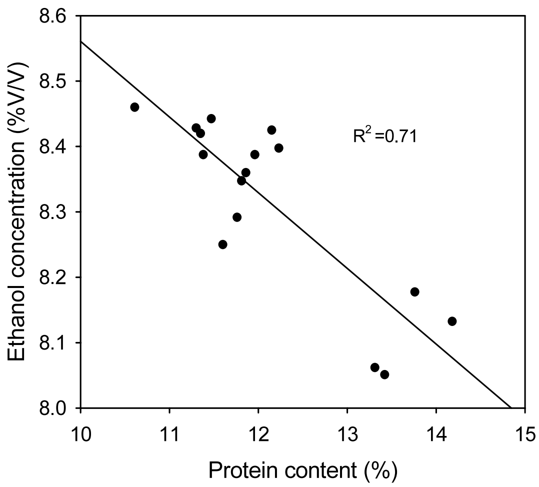

Fig. 4. Effect of protein content on ethanol production.

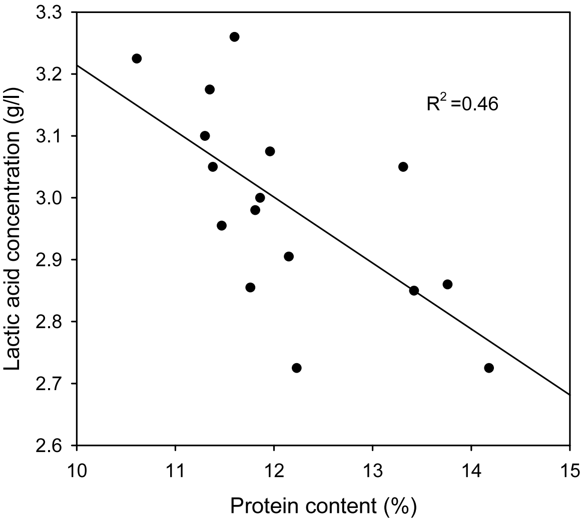

Fig. 5. Effect of protein content on lactic acid production.

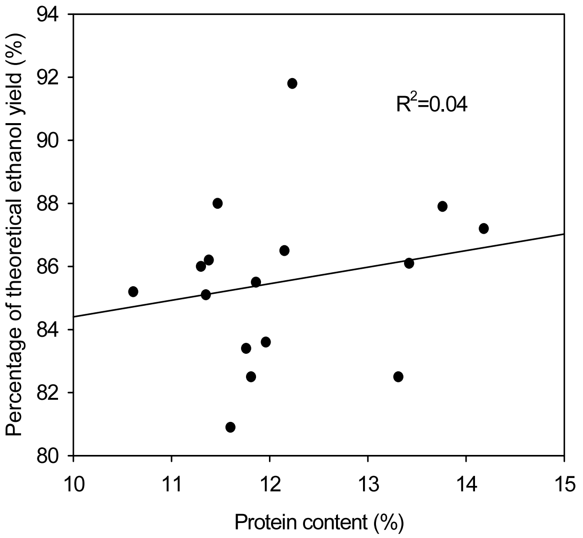

Fig. 6. Effect of protein content on percentage of theoretical ethanol yield.

4. Conclusions

Fermentation studies were used to evaluate the effect of sorghum genotype and growth environment. Both sorghum genotype and production environment had significant effects on ethanol and lactic acid yields. The effect of production environment on the fermentation process was 5 and 10% for ethanol and lactic acid yields, respectively. Variations of 5% for ethanol and 15% for lactic acid yields were observed among the 16 sorghum samples. The effect of genotype and environment on ethanol and lactic acid production is related to both the chemical composition and physical properties of the sorghum grain samples, with a stronger effect observed for chemical composition. Ethanol and lactic acid production increased as starch content increased, whereas the ethanol and lactic acid production decreased as protein content increased. Protein content had no significant effect on the percentage of theoretical conversion rate for ethanol fermentation. Chemical composition had a greater effect on the ethanol and lactic acid yields than physical properties of the sorghum kernels. The effect of physical properties on ethanol and lactic acid yields was not significant ($P > 0.05$).

Further research is needed to test a broad number of varieties across a wide range of growing conditions to further evaluate the effects on ethanol and lactic acid fermentation yields.

Acknowledgements

Mention of firm names or trade products does not constitute endorsement by the US.

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